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Effect of photobiomodulation on salivary flow and composition, xerostomia and quality of life of patients during head and neck radiotherapy in short term follow-up: A randomized controlled clinical trial

Gabriel Campos Louzeiro, Karen Cherubini, Maria Antonia Zancanaro de Figueiredo, Fernanda Gonçalves Salum*

School of Health and Life Sciences, Oral Medicine Division, Pontifical Catholic University of Rio Grande do Sul- PUCRS, Porto Alegre, Rio Grande do Sul, Brazil

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ABSTRACT

Xerostomia and hyposalivation are frequent conditions in patients undergoing head and neck radiotherapy, which usually lead to a worsening of quality of life. This study aimed to assess whether photobiomodulation (PBM) can minimize hyposalivation, xerostomia and qualitative changes on saliva and improve quality of life in patients undergoing radiotherapy in short-term follow-up. Twenty-one patients were randomly divided into two groups: sham group (SG) and laser group (LG). A diode laser was used for intra- (660 nm, 10 J/cm^2, 0.28 J per point, 40 mW) and extra-oral (810 nm, 25 J/cm^2, 0.7 J per point, 40 mW) applications over the salivary glands, three times a week, during the entire radiotherapy period. In SG, the tip of the instrument was sealed with blue rubber to prevent the passage of light. Xerostomia and pH were evaluated and unstimulated and stimulated salivary flow was determined before the start of radiotherapy (T1), after the 15th session (T2), after the end of radiotherapy (T3) and 60 days after radiotherapy (T4). Concentrations of calcium, total proteins, chloride, sodium, potassium and amylase and catalase activities were evaluated in stimulated saliva samples. Quality of life was assessed at times T1 and T4. Generalized estimating equations were used to assess differences in the outcome between times and groups. All patients showed worsening in unstimulated ($p = .003$) and stimulated ($p < .001$) salivary flow, xerostomia ($p < .05$) and quality of life during radiotherapy ($p = .001$). An increase in chloride concentrations was observed at times T3 and T4 ($p < 0,05$), and a reduction in amylase activity at T3 ($p < .05$). Unstimulated saliva pH was higher in LG than SG at T3 ($p = .037$). No difference between groups was noted in relation to salivary flow and composition, xerostomia or quality of life. Our results suggest that PBM may help in preserving salivary pH during radiotherapy.

1. Introduction

Radiotherapy is a well-established cancer treatment modality for malignant neoplasms of the head and neck. It uses the emission of ionizing radiation in doses of 50 to 70 Gray (Gy) over the tumor area [1], divided into daily fractions ranging from 1.8 to 2 Gy [2]. Its mechanism of action is based on the induction of cellular apoptosis by direct damage of DNA structure or indirectly by the production of free radicals generated by hydrolysis of water molecules. Due to the greater integrity of the cell control mechanism, non-tumor cells tend to be less sensitive to radiation compared to neoplastic cells; however, the toxicity to structures adjacent to the tumor depends on the degree of cell division. Cells with a high potential for division are more sensitive to ionizing radiation [3].

Because of the low degree of cell division, it is expected that salivary glands are resistant to ionizing radiation, but these structures seem to be highly radiosensitive [4–7]. Salivary glands show significant loss of function in the first weeks of radiotherapy, which worsens during treatment in a dose-effect relationship. Saliva becomes more viscous with changes in its composition. In normal circumstances, saliva produced in acini is isotonic, showing a similar composition of sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-) in plasma [8–11]. As it flows through ductal cells, Na^+ and Cl^- are absorbed by cells and K^+ and HCO_3^- are secreted into saliva, giving it hypotonicity when compared to plasma [9–12]. During radiotherapy, there is a decrease in HCO_3^- concentration, associated with an increase

* Corresponding author at: School of Health Science, Pontifícia Universidade Católica do Rio Grande do Sul, São Lucas Hospital, Av. Ipiranga 6690, Porto Alegre, RS CEP: 90610-000, Brazil.
E-mail address: fernanda.salum@pucrs.br (F.G. Salum).

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in acidity [13] and an increase in protein and electrolyte concentrations such as Na^+, K^+ and Ca^{2+} [14–16], caused by permeability changes due to damage to glandular structures. These changes considerably reflect the loss of salivary properties and function [7]. As a consequence, head and neck radiotherapy patients often develop dysphagia, dysphonia, loss of taste and opportunistic infections, which causes considerable loss of quality of life [7,17–20].

Photobiomodulation (PBM) is a therapeutic modality capable of altering biological activity by photon energy. Light energy interacts with the irradiated tissue, inducing non-thermal effects that can assist in the process of tissue repair, pain relief and inflammatory control [21]. Due to these effects, this modality has been widely approached to control the side effects of cancer treatment, especially in the treatment of oral mucositis [22–25]. More recently, PBM has been studied in the treatment of salivary gland disorders [26,27]. Animal studies have shown that PBM is not only able to stimulate salivary flow, but it also regulates salivary composition and controls the redox mechanism of the glands, reducing the local inflammatory process [28–30]. In a clinical context, PBM has been shown to be able to minimize hyposalivation when used concurrently with head and neck radiotherapy [31–36].

PBM is a promising approach to the management of head and neck radiotherapy side effects, but there is still little evidence that it controls ionizing radiation-induced hyposalivation and xerostomia. There is no consensus on its effects when used concurrently with radiotherapy. Although many studies have shown significant increases in salivary flow, it is not yet known whether this increase reflects a radioprotective mechanism and whether this increase can have repercussions in a clinical setting, as most patients suffer from considerable loss of quality of life. Given this, the present study aimed to evaluate the effect of PBM on salivary flow, xerostomia, salivary composition and quality of life of patients undergoing head and neck radiotherapy in short-term follow-up.

2. Materials and Methods

2.1. Ethical Consideration

This study was previously approved by the Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (protocol No. 1.892.495). Participants were informed about the objectives and procedures of the study, and then read and signed an informed consent form.

2.2. Study Design

Twenty-seven patients with malignant head and neck cancer participated in this study. They were referred from the Radiotherapy Department of the São Lucas Hospital of the Pontifical Catholic University of Rio Grande do Sul (PUCRS) to the Stomatology Department of the hospital, with indication for chemoradiotherapy or radiotherapy. All patients were irradiated in the head and neck region using a linear accelerator (Clinac® IX system or Trilogy® system) with a minimum dose of 50 Gy over the tumor area, encompassing at least one parotid and one submandibular salivary gland. We included patients of both sexes, over 18 years old, not previously submitted to head and neck radiotherapy, and who had a Karnofsky performance scale higher than 60. Excluded from this study were patients with malignant neoplasms of hematological origin, reactions or restrictions with the use of benzydamine hydrochloride or PBM, who had collagen disease, Sjögren's syndrome or diabetes, and those who that did not agree to participate or did not sign an informed consensus form.

All patients received an oral examination and salivary gland function evaluation. Dental extraction, periodontal and restorative treatments were performed according to each individual's needs before radiotherapy was started. The patients were followed three times a week throughout the radiotherapy period and were instructed to maintain thorough oral hygiene, and to avoid hot or spicy beverages and foods, as well as toothpastes or solutions that may cause mouth burning. An antifungal agent was given in case of the appearance of oral candidiasis. To minimize possible radiation-induced mucositis, benzydamine hydrochloride (15 mg tablets) were given to every participant to use four times a day during radiotherapy.

The patients were randomly divided in two defined groups: Laser Group (LG) and Sham Group (SG). The division was performed using the Research Randomizer platform (www.randomizer.org). Each patient was numbered according to the order of participation and randomly allocated according to the software tool. The patients did not know which group they were allocated to.

2.3. Laser Therapy

An indium-gallium-aluminum phosphide diode laser (Photon Lase III - DMC® Ltda, São Carlos, SP, Brazil), with spot size of 0.028 cm^2 was applied in LG. For the major salivary glands, the laser was operated at 810 nm (AsGaAl), in continuous waves, in contact mode, 40 mW power output, at a fluence of 25 J/cm^2 (equipment fluence) corresponding to 17.5 s of exposure time and 0.7 J per point [26,28,29,32,35,37]. The laser was applied at the following sites: 6 points extraorally for each parotid gland, 3 points extraorally for submandibular glands bilaterally and 2 points intraorally on anterior region of mouth floor according to each sublingual gland. For minor salivary glands, the laser was operated at 660 nm (InGaAlP), in continuous waves, in contact mode, 40 mW power output, at a fluence of 10 J/cm^2 (equipment fluence), corresponding to 7 s of exposure time and 0.28 J per point [30,32–35]. The laser was applied at the following sites: 1 point on each labial commissure, 8 points on superior labial mucosa, 8 points on inferior labial mucosa, 12 points on each buccal mucosa, 12 points on hard palate, 4 points on soft palate, 6 points on each border of the tongue, 6 points on ventral surface of the tongue and 4 points on mouth floor. Laser radiation over tumor was avoided by striking at least 1 cm from the area. In case of tumor infiltration into the major salivary glands, this gland did not receive laser therapy.

In the SG, the same laser protocol was used, except that the instrument tip was sealed with blue rubber to prevent any laser radiation of the tissue. To ensure correct dosimetry in the groups, the power of the equipment was checked before each application with a power meter (Laser Check – MMO® Ltda., São Carlos, SP, Brazil). In case of loss of more than 20% of power (according to manufacturer specifications) for LG, it was set to 50 mW and the energy density adjusted to 31 J/cm^2 for 810 nm wavelength and 13 J/cm^2 to 660 nm wavelength. In SG patients, the measured power did not exceed 0 mW. In both groups the application started immediately after the first radiotherapy session three times a week, preferably on alternate days, until the last radiotherapy session.

2.4. Salivary Flow and pH Assessment

Stimulated and unstimulated whole saliva samples were collected from all patients in 4 periods: before the first radiotherapy session (T1), at 15th radiotherapy session (T2), at the last radiotherapy session (T3) and after 2 month (T4). Salivary samples were collected in the morning, between 8:00 and 11:00 h to avoid variations due to the circadian cycle, and all patients were instructed to eat as usual but to avoid food and drinks for one hour before the examination. Prior to collection, the patients rinsed their mouth with distilled water for 30 s to reduce the accumulation of organic matter, and were placed in a room with adequate light, ventilation and temperature.

For whole unstimulated saliva collection, the patients were asked to swallow all the saliva present in the mouth and lean forward without further swallowing. The patients were asked to accumulate saliva on the mouth floor and spit into a 50-mL Falcon tube previously weighed with a precision balance and stored in a low temperature Styrofoam

container, every 60 s for 15 min, where a sterile funnel was used for saliva collection.

For stimulated whole saliva collection, patients were instructed to discard the initial saliva and chew a piece of latex measuring 1 × 1 cm attached to a piece of floss to prevent accidental swallowing of the material. The patients were expected to expel saliva into a sterile 50 mL falcon tube previously weighed with a precision balance and stored in a low temperature Styrofoam container, aided by a sterile funnel, for 15 min.

To calculate the volume of saliva expelled, the samples were weighed on a precision balance and the result obtained was subtracted from the weight of the tube used. Salivary flow velocity was obtained by dividing the volume by the collection time. Each gram was considered to be equal to 1 mL of saliva. The samples were then immediately taken to the laboratory and the pH determined with a bench pH meter model 3510 (Jenway, Staffordshire, UK). Stimulated salivary samples were centrifugated (14 *g* for 10 min) and the supernatant was separated into 2 mL Eppendorf tubes, frozen and stored at −80 °C until further analysis.

2.5. Xerostomia Assessment

Xerostomia was analyzed at T1, T2, T3 and T4 by two questionnaires: Visual Analog Scale (VAS) and Treatment-Emergent Symptom Scale (TESS).

VAS has eight major topics, and each topic has a horizontal line 10 cm long. The values are comprised between zero (meaning absence of symptom) and ten (maximum symptomatology). The patient was instructed to mark a vertical line within each line. Symptomatology quantification was performed by analysis in centimeters between the beginning of the line and the demarcation of the line made by the patient.

TESS is a scale that uses the frequency of xerostomia, based on the following scores: 0 - no complaints; 1 - Light; 2 - Smooth; 3 - Moderate; and 4 - Severe. Higher scores are associated with worse symptoms.

2.6. Quality of Life Assessment

Quality of life was assessed at T1 and T4 using the University of Washington Quality of Life Questionnaire (UW-QOL). This questionnaire has twelve domains of questions (pain, appearance, activity, recreation, swallowing, chewing, speech, shoulder, taste, saliva, mood and anxiety) with three to six answer alternatives for each domain. Each alternative has different scores ranging 0–100, with lower scores indicating poorer quality of life.

2.7. Salivary Quality Analysis

Protein concentration in stimulated saliva samples was determined by the biuret method [38] (Labtest Diagnóstica, Lagoa Santa, Brazil). A 4 g/dL bovine serum albumin and 14.6 mmol/L sodium azide were used as standard. The concentration was determined by the absorbance read on a spectrophotometer (Genesys 10s UV-VIS – Hexis Científica, Jundiaí, Brazil) at 660 nm.

Salivary calcium was measured by the colorimetric method [39] (Labtest Diagnóstica, Lagoa Santa, Brazil). This technique is based on a spectrophotometer (Genesys 10s uv-vis – Hexis Científica, Jundiaí, Brazil) reading (570 nm) of the reaction between calcium and purple phthalein (o-cresolphthalein complexone 320 μmol/L; 8-hydroxyquinoline 13 mmol/L and hydrochloric acid 130 mmol/L) in alkaline medium (920 mmol/L buffer, pH 12). A 10 mg/L calcium was used as standard.

Amylase activity was evaluated by the modified Caraway method [40] (Labtest Diagnóstica, Lagoa Santa, Brazil). The samples were diluted 600× in pure water (Milli-Q) and blotted with a starch substrate (0.4 g/dL in pH 7 phosphate buffer), which after the addition of iodine reagent (potassium iodate 16.7 mmol/L; potassium iodide 271 mmol/L and hydrochloric acid 112 mmol/L), decreased the blue color. The color difference between substrate and control is proportional to amylase activity and can be measured at 660 nm (Genesys 10s UV-VIS – Hexis Científica, Jundiaí, Brazil).

The concentration of chloride ions was determined by the mercury thiocyanate colorimetric method [41] (Labtest Diagnóstica, Lagoa Santa, Brazil). Stimulated salivary samples were placed in 1 mL of mercury thiocyanate reagent (2 mmol/L mercury thiocyanate, mercury chloride 0.8 mmol/L, ferric nitrate 20 mmol/L, 28 mmol/L nitric acid and stabilizer). The reaction between chlorine and mercury thiocyanate produces thiocyanate ions which when combined with iron ions produces iron thiocyanate. Iron thiocyanate can be measured at 450 nm (Genesys 10s UV-VIS – Hexis Científica, Jundiaí, Brazil), and its absorbance is proportional to chloride concentration, and 100 mEq/L chloride was used as standard.

Salivary potassium and sodium were measured by enzymatic reaction (Labtest Diagnóstica, Lagoa Santa, Brazil). Potassium concentration was determined by adding salivary samples to phosphoenolpyruvate substrate (lactate dehydrogenase 50 KU/L; phosphoenolpyruvate 100 mmol/L; NADH 10 mmol/L; ADP 100 mmol/L and lithium azide 0.095%) and incubating samples at 36° for 5 min. After adding pyruvate kinase 50 KU/mL in a cuvette at 36 °C, phosphoenolpyruvate is converted to pyruvate. Pyruvate is then converted to lactate in the presence of NADH, catalyzed by lactate dehydrogenase. The optical density reduction is evaluated after 2 and 4 min in a 380 nm spectrophotometer (Spectramax M2 - Molecular Devices, San Jose, CA, USA), which is proportional to the potassium concentration in the sample. For sodium concentration, the addition of salivary sample activates the reaction between the sodium-dependent enzyme beta-D-galactosidase (0.4 mM cryptand; beta-D-galactoside 80 U/mL; 0.02% isothiazolone in 50 mM pH 8.5 buffer) and its ONPG substrate (ONPG 0.5 mM; isothiazolone 0.02% in buffer 50 mM, pH 6.5), converting the substrate to *O*-nitrophenyl and galactose. The rate of formation of the product is proportional to the sodium concentration, which can be recorded in cuvettes at 36 °C, at 1 and 3 min after the beginning of the reaction in a spectrophotometer calibrated at 405 nm (Spectramax M2 - Molecular Devices, San Jose, CA, USA).

Catalase activity was evaluated as described elsewhere [42]. The reagent was prepared from the 50 μl ultrasound mixture of Triton-x 100 in 50 mL of distilled water. After homogenization, 51 μl of 30% hydrogen peroxide was added to the reagent. Peroxidase activity was evaluated by adding 20 μl of salivary samples in 1.98 mL of reagent and recording absorbance in a spectrophotometer calibrated at 240 nm every 30 s for 1 min. The difference between final and initial absorbance is proportional to the catalase activity according to the total amount of protein in the sample.

2.8. Statistical Analysis

Demographic characteristics, comorbidities, medications, and categorical data of tumor and radiotherapy (histological type, staging, location, tumor dose, surgery and chemotherapy) were summarized as descriptive statistics in Tables 1 and 2.

The Student *t*-test was used to compare radiation dose differences in parotid and submandibular glands between groups. Differences in salivary flow, salivary pH, salivary composition, xerostomia and quality of life were evaluated using generalized estimating equations, complemented by the Bonferroni test. The significance level was set at 5%.

The sample size was determined using G*Power software. A medium effect size of 0.25 with $\alpha = 0.05$ and power = 0.80, resulted in an estimated size of $N = 28$.

3. Results

During the study experiment, six patients needed to be excluded

Table 1
Demographic characteristics, comorbidities and medications of laser and sham groups.

	Laser group		Sham group		Total	
	N	%	N	%	N	%
Gender						
Male	7	70	9	81.82	16	76.19
Female	3	30	2	18.18	5	23.81
Age						
48–59	4	40	2	18.18	6	28.57
60–69	5	50	4	36.36	9	42.86
≥70	1	10	5	45.45	6	28.57
Comorbities						
Renal diseases	1	10	1	9.09	2	9.52
Cardiovascular diseases	7	70	8	72.73	15	71.43
Osteoporosis	1	10	0	0	1	4.76
Hypothyroidism	1	10	0	0	1	4.76
Depression	0	0	1	9.09	1	4.76
Medications						
Statins	3	30	1	9.09	4	19.05
Antihypertensive	4	40	11	100	15	71.43
Levothyroxine	1	10	0	0	1	4.76
Pain killers	0	0	3	27.27	3	14.29
Antidepressants and anxiolytics	1	10	2	18.18	3	14.29
Proton pump inhibitors	1	10	0	0	1	4.76
AAS	0	0	2	18.18	2	9.52
Antiosteoporotic	1	10	0	0	1	4.76
Tibolone	1	10	0	0	1	4.76
Diosmin	0	0	1	9.09	1	4.76
Metadoxil	1	10	0	0	1	4.76

Table 2
Radiation dose differences in the parotid (PAR) and submandibular (SMG) glands between the laser and sham groups.

	Laser group		Sham group		*p*
	Mean dose (Gy)	Std dev.	Mean dose (Gy)	Std dev.	
Right PAR	47.38	22.006	45.91	18.636	0.877
Left PAR	36.56	26.501	48.70	16.159	0.239
Right SMG	59.00	20.188	64.09	14.074	0.507
Left SMG	48.40	25.812	65.27	8.063	0.074

Comparisons between mean salivary gland doses using the Student *t*-test ($p \leq .05$).

from the sample for the following reasons: two patients could not perform morning evaluations at T2 and T3 due to time mismatch problems; one patient requested removal before T2; the radiation dose did not reach the salivary glands in two patients; one patient was diagnosed with diabetes. Final sampling resulted in 21 patients, 10 allocated in LG and 11 in SG. All patients were over 47 and under 81 years old (mean = 64.05 ± 8.33), with predominance of males in both groups. The distribution of demographics characteristics, medications and comorbidities in the groups are presented in Table 1. No statistically significant difference was observed in dose cover in parotid and submandibular salivary glands between LG and SG (Table 2). Table 3 shows variables according to tumor, radiotherapy dose and other oncologic treatment characteristics in each group.

One patient of LG at T3 and two patients of each group at T4 showed no saliva flow (for both unstimulated and stimulated saliva). Regardless of study groups, the mean unstimulated salivary flow at T3 significantly declined compared to T1 ($p = .003$) and T2 ($p = .035$). A slight decrease was also shown between T3 and T4, which was not significant ($p = .140$). There were no statistically significant differences between LG and SG at T2, T3 and T4 ($p > .05$). In stimulated salivary flow, the mean rate at baseline decreased significantly at T2 ($p < .001$). Also, T4 mean rate decreased compared to T2 ($p = .001$). At T1, SG exhibited higher mean stimulated flow rate compared to LG

Table 3
Characteristics of tumor and treatment between laser and sham groups.

	Laser group		Sham group		Total	
	n	%	n	%	n	%
Histological type						
Squamous cells carcinoma	7	70	10	90.91	17	80.95
Adenocarcinoma	2	20	1	9.09	3	14.29
Osteosarcoma	1	10	0	0	1	4.76
Staging						
Stage II	1	10	0	0	1	4.76
Stage III	3	30	5	45.45	8	38.09
Stage IV	6	60	6	54.54	12	57.14
Location						
Palate	0	0	2	18.18	2	9.52
Larynx	0	0	4	36.36	4	19.05
External auditory canal	1	10	0	0	1	4.76
Tonsillar pillar	1	10	1	9.09	2	9.52
Mandible	1	10	0	0	1	4.76
Floor of the mouth	1	10	0	0	1	4.76
Tongue	1	10	2	18.18	3	14.29
Oropharynx	1	10	0	0	1	4.76
Parotid	2	20	1	9.09	3	14.29
Occult primary tumor	2	20	1	9.09	3	14.29
Tumor dosage (Gy)						
50–59	1	10	0	0	1	4.76
60–69	3	30	6	54.54	9	42.86
≥70	6	60	5	45.45	11	52.38
Previous surgery						
Yes	4	40	2	18.18	6	28.57
No	6	60	9	81.82	15	71.43
Chemotherapy protocol						
DDP*	6	60	6	54.54	12	57.14
DCF**	0	0	1	9.09	1	4.76
No chemotherapy	4	40	4	36.36	8	38.09

* Cisplatin.
** Docetaxel, Cisplatin and 5-fluorouracil.

($p = .029$), and no additional differences were observed between groups over time (Fig. 1).

No difference between groups was observed in quality of life by the UW-QOL, in either T1 or T4 period. On the other hand, there was a significant decrease in mean score of quality of life among the study population between T1 and T4 (Fig. 2). Regarding xerostomia, the same effect was seen. Tess and VAS showed no differences between groups, but worsening of symptoms was observed from the 15th session of radiotherapy on both questionnaires (Figs. 3 and 4).

Because of asialia seen in one patient at T3 and in four patients at T4, no sialochemistry could be performed in these samples. In addition, due to saliva consistency or scarcity, total protein, calcium, chloride, sodium, potassium concentration and catalase and amylase activity could not be evaluated in a SG patient at T3, and sodium concentration could not be evaluated in a LG patient at T3. Overall chloride concentration showed a significant increase at T3 ($p < .001$), which remained elevated until T4 ($p = .015$). Meanwhile, amylase activity decreased significantly between T1 and T3 ($p < .05$), followed by a slight but not significant increase at T4 (Table 4). No differences in total protein, calcium, sodium, potassium concentration and catalase activity were observed between LG and SG in any period (Table 5). In LG, a higher pH value was observed at T3 ($p = 0{,}037$) in unstimulated saliva, compared to SG. No differences were observed in stimulated salivary pH (Fig. 5).

4. Discussion

It seems that PBM can activate photoreceptors in the respiratory chain, leading to an increase in ATP production. As a consequence, Ca^{2+} ATPase pumps increase intracellular calcium concentration, and ATP also induces cAMP formation. Thus, the effect of PBM over the salivary gland is expected to act in favor of nervous stimuli by

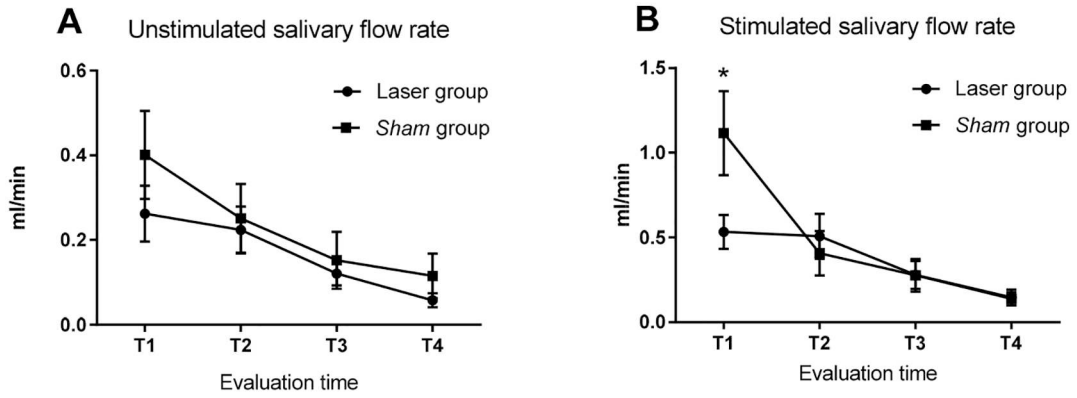

Fig. 1. Graphs comparing mean (± SD) unstimulated (graph A) and stimulated (graph B) salivary flow rate (mL/min) between laser and sham group at baseline (T1), 15th session (T2), final session (T3) and 60 days (T4) after radiotherapy treatment (*p = .029).

increasing salivary flow rate [43,44]. In addition, Karu, Pyatibrat & Kalendo [45] described that PBM also confers a radioprotective effect over the cells under ionizing radiation. In this way, clinical trials have suggested that PBM could minimize hyposalivation by increasing salivary flow.

In the present study, sialometric, sialochemical and subjective evaluations were performed to determine whether PBM can minimize salivary gland dysfunction and whether these changes can influence the quality of life of radiotherapy patients.

Our results showed a decrease in salivary flow rate over time, in all patients. A significant decrease in salivary flow rate was seen immediately after radiotherapy for unstimulated saliva, at the 15th radiotherapy session for stimulated saliva. However, in this study, PBM did not improve salivary flow rate. No significance difference in both unstimulated and stimulated salivary flow rate was observed between laser and control groups. These results differ from those obtained by Simões et al. [33], Gonnelli et al. [32,35], Libik et al. [31], Lopes, Mas & Zângaro [34] and Oton-Leite et al. [36], in which the unstimulated and stimulated salivary flow rate gradually increased in PBM patients between the 15th session and 30 days after radiotherapy, compared with other forms of control. On the other hand, only one study [36] was randomized and blinded, while the others were not. In our study, rigorous methodological control was performed, ensuring adequate blinding and randomization of all participants, and even so, the laser did not show an effect on the salivary flow. However, some methodological approaches distinguish our study from the others.

First, we chose the mechanical method of salivary stimulation, instead of the gustatory method as indicated [46]. Despite showing a lower stimulating power than the gustatory stimulus, this method does not use chemical substances which may interfere in the analysis of salivary components [47]. According to Saavedra et al. [48], the use of citric acid as a taste stimulant causes a significant decrease in pH values during the first minute of collection, thereby changing the actual values of analyses. Also, a methodological approach similar to ours [49] demonstrated that salivary flow variations measured with the chewing technique are proportional to the variations of xerostomia symptom and quality of life in patients under head and neck radiotherapy, suggesting that the masticatory technique is a good method to correlate with subjective analysis. In addition, the choice of the stimulation method does not explain the fact that PBM did not alter unstimulated salivary flow.

Second, since many patients show considerable salivary flow reduction at the end of radiotherapy treatment, the salivary collection time was extended to 15 min to obtain a greater amount of saliva to evaluate the qualitative properties. It is not clear whether increased collection time may lead to variations in salivary flow average. However, our findings in the laser group showed similarities in values with the results obtained by Oton-Leite et al. [36] and Simões et al. [33], who set the collection time at 5 min and 10 min, respectively. In contrast, large variations in salivary flow values after PBM have been observed in several studies [31,34,36]. This may possibly be the result of different PBM protocols used or variations in participant characteristics.

It is worth noting that salivary flow shows great personal variation [50]. In addition, the debilitating state caused by tumor seems to significantly influence the reduction of salivary flow [51]. These are possible explanations of why the control group had a significantly higher value of stimulated salivary flow at baseline. A marked reduction in stimulated salivary flow was observed in the SG in the 15th session of radiotherapy. This significant reduction in salivary flow in the first two weeks, seems to be a normal reaction to radiotherapy [52]. This effect does not seem to have occurred in the LG. In this group, the

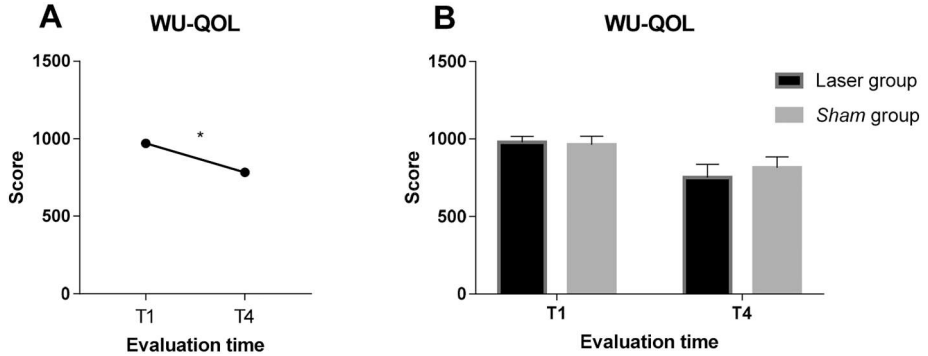

Fig. 2. Graphs comparing mean (± SD) quality of life score at baseline (T1) and 60 days (T4) after radiotherapy in the study population (graph A) and between groups (graph B) (*p = .001).

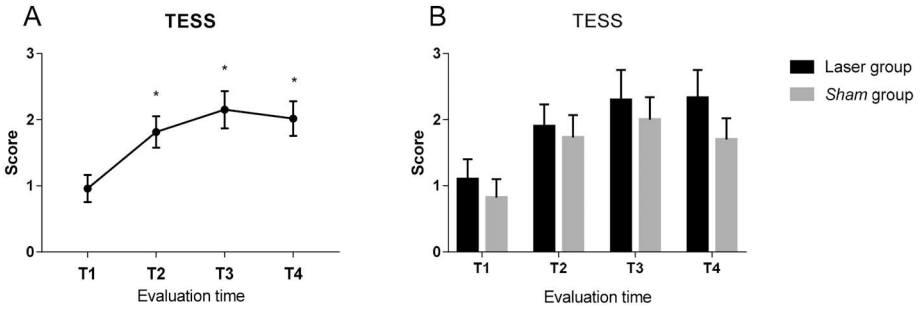

Fig. 3. Graphs comparing mean (± SD) TESS score at baseline, 15th session, final session and 60 days after radiotherapy in the study population (graph A) and between groups (graph B) (*$p < .05$).

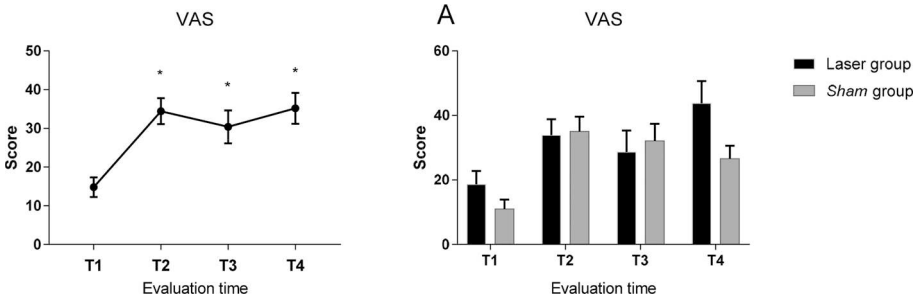

Fig. 4. Graphs comparing mean (± SD) VAS score at baseline (T1), 15th session (T2), final session (T3) and 60 days (T4) after radiotherapy treatment the study population (graph A, *$p < .05$) and between groups (graph B).

Table 4
Sialochemical comparisons between evaluation times.

Outcome	Evaluation time			
	T1	T2	T3	T4
Total protein concentration (g/dL)	0.37 (± 0.11)^{A}	0.28 (± 0.06)^{A}	0.47 (± 0.15)^{A}	0.45 (± 0.13)^{A}
Calcium concentration (mg/dL)	6.56 (± 0.44)^{A}	7.65 (± 1.09)^{A}	10.53 (± 3.48)^{A}	7.98 (± 0.65)^{A}
Chloride concentration (g/dL)	25.76 (± 2.92)^{B}	36.91 (± 4.70)^{AB}	49.95 (± 7.00)^{A}	47.39 (± 7.87)^{A}
Sodium concentration (mmol/L)	22.87 (± 3.61)^{A}	21.82 (± 3.21)^{A}	28.21 (± 5.42)^{A}	28.28 (± 6.50)^{A}
Potassium concentration (mmol/L)	15.96 (± 0.78)^{A}	16.20 (± 1.07)^{A}	17.43 (± 2.87)^{A}	16.18 (± 1.83)^{A}
Amylase activity (U/dL *10^{4})	11.64 (± 1.82)^{A}	11.88 (± 2.74)^{AB}	6.93 (± 1.08)^{B}	7.81 (± 1.49)^{AB}
Catalase activity (mmol/min *10^{-3})	8.69 (± 2.53)^{A}	5.19 (± 1.47)^{A}	4.71 (± 1.38)^{A}	6.61 (± 1.95)^{A}

Values expressed as mean and standard deviation. Different letters in columns represent differences according to different times in all studied patients (generalized estimating equations, complemented by Bonferroni's test).

Table 5
Sialochemical comparisons between laser and sham groups according to evaluation time.

Outcome	Group	Evaluation time			
		T1	T2	T3	T4
Total protein concentration (g/dL)	Laser	0.24 (± 0.06)^{Aa}	0.35 (± 0.10)^{Aa}	0.45 (± 0.27)^{Aa}	0.45 (± 0.25)^{Aa}
	Sham	0.51 (± 0.20)^{a}	0.21 (± 0.05)^{a}	0.48 (± 0.14)^{a}	0.46 (± 0.04)^{a}
Calcium concentration (mg/dL)	Laser	6.05 (0.42 ±)^{Aa}	8.09 (± 1.92)^{Aa}	13.31 (± 6.87)^{Aa}	7.15 (± 0.93)^{Aa}
	Sham	7.07 (± 0.77)^{a}	7.20 (± 1.01)^{a}	7.75 (± 1.14)^{a}	8.80 (± 0.92)^{a}
Chloride concentration (g/dL)	Laser	25.38(± 2.97)^{Aa}	39.63(± 7.76)^{Aa}	45.04 (± 10.69)^{Aa}	41.94 (± 11.64)^{Aa}
	Sham	26.14 (± 5.02)^{a}	34.18 (± 5.31)^{a}	54.86 (± 9.05)^{a}	52.84 (± 10.61)^{a}
Sodium concentration (mmol/L)	Laser	24.34(± 6.38)^{Aa}	19.35(± 4.74)^{Aa}	23.29 (± 6.90)^{Aa}	36.97 (± 11.59)^{Aa}
	Sham	21.40 (± 3.39)^{a}	24.28 (± 4.33)^{a}	33.13 (± 8.36)^{a}	19.59 (± 5.90)^{a}
Potassium concentration (mmol/L)	Laser	16.03 (± 1.27)^{Aa}	16.75(± 1.93)^{Aa}	21.44 (± 5.58)^{Aa}	16.30 (± 3.13)^{Aa}
	Sham	15.90 (± 0.92)^{a}	15.64 (± 0.91)^{a}	13.41 (± 1.36)^{a}	16.06 (± 1.89)^{a}
Amylase activity (U/dL*10^{4})	Laser	10.40 (± 2.22)^{Aa}	11.87(± 5.05)^{Aa}	6.21 (± 1.44)^{Aa}	6.03 (± 1.84)^{Aa}
	Sham	12.88 (± 2.88)^{a}	11.90 (± 2.12)^{a}	7.65 (± 1.60)^{a}	9.60 (± 2.36)^{a}
Catalase activity (mmol/min*10^{-3})	Laser	6.22 (± 2.76)^{Aa}	5.79 (± 1.98)^{Aa}	1.71 (± 0.56)^{Aa}	5.55 (± 2.88)^{Aa}
	Sham	11.16 (± 4.24)^{a}	4.59 (± 2.17)^{a}	7.70 (± 2.70)^{a}	7.68 (± 2.63)^{a}

Values expressed as mean and standard deviation followed by upper case letters in different columns to compare differences according to time and lower case letters to compare differences between groups (generalized estimating equations, complemented by Bonferroni's test).

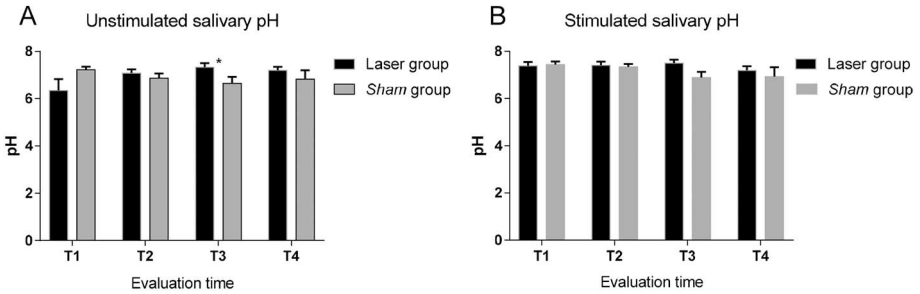

Fig. 5. Graphs comparing mean (± SD) unstimulated (graph A) and stimulated (graph B) salivary pH between laser and sham group at baseline (T1), 15th session (T2), final session (T3) and 60 days (T4) after radiotherapy (*p = .037).

reduction in stimulated salivary flow was discreet, gradual and it was significantly lower, compared to the base period, only in 60 days of radiotherapy. It is not clear whether this pattern is related or not to the biostimulatory effect of the laser. The values of both groups tended to approach over the course of radiotherapy. Given this, the possibility that PBM would have caused radiation protection to the LG should also not be ruled out.

According to the qualitative saliva analyses, PBM gradually increased unstimulated salivary pH during radiotherapy, showing significant difference with the control group at the end of radiotherapy treatment. These results corroborate those of Palma [35], who observed an increase in pH in patients previously irradiated in the head and neck region. Due to the biomodulatory activity of laser therapy, we hypothesized that PBM may exert a radioprotective effect on striatal cells of salivary ducts. These cells are responsible for absorbing salivary chlorine while secreting bicarbonate [12]. Radiotherapy alters the activity of these cells, increasing chlorine concentration and decreasing bicarbonate concentration of whole saliva [13,15,53,54]. As observed, the average chlorine concentration in the patients in this study increased as the radiation accumulated, showing damage to the salivary ducts. PBM may be able to reduce this damage to these cells, allowing greater saliva buffering activity, although no changes in salivary chloride concentration were observed. Interestingly, the salivary pH value in the laser group tended to increase even with radiation accumulation, while the control group showed a gradual but not significant reduction. This increase in salivary pH may be beneficial in maintaining the patient's oral homeostasis, since the decrease in pH induces changes in oral microflora [55,56], increases the risk of developing opportunistic infections [7] and compromises dental integrity [57].

It is important to note that this study is limited to a short evaluation period. Although an increase in unstimulated salivary pH was observed, a tendency towards a reduction in stimulated and unstimulated salivary pH was observed in T4. This evidence is not sufficient to predict the durability of the effects of photobiomodulation. Since radioinduced salivary dysfunctions are mostly irreversible and accompany the patient throughout life, the effects of PBM on salivary pH should not be interpreted over long periods.

Despite duct radioprotection by PBM, the same effect was not observed with acinar cells. Alpha amylase is a great secretory marker of acini [58], and the drop in its activity during ionizing radiation has been previously described [59]. Acinar damage was observed as a reduction in amylase activity in all patients, but PBM was not shown to increase this activity.

There was no significant difference between the groups in total protein, calcium, sodium and potassium concentration. In contrast, other studies have observed an increase in total protein [15,16,59,60], sodium [14,15,54], potassium [13,16] and calcium [13,15,16] concentration during radiotherapy as consequences of damage to acini and duct cells. In addition, PBM was not shown to alter catalase activity. It is known that one of the effects of PBM is to modulate the antioxidant system. According to Zecha et al. [25], this is one of the possible explanations of the beneficial effects of PBM in the management of complications resulting from radiotherapy. Few studies have shown that PBM may reduce catalase activity in salivary glands of diabetic animals [29,61], due to local inflammatory modulation. Despite that, PBM does not appear to have an effect on catalase activity in saliva of patients undergoing radiation therapy [33].

Regarding xerostomia, some evidence demonstrates a small increase salivary flow rate of patients treated with PBM [31,32,34–36]. Although, there is no proof that this increase can result in improvement in xerostomia in radiotherapy patients. In a study [33], patients that received intraoral applications of laser 660 nm three times a week did not show a decrease in xerostomia symptoms; however, this study was not controlled, therefore, it had limitations in the evaluation of the laser effect. In our study, VAS and TESS questionnaires showed no difference in xerostomia symptom improvement using PBM. Besides that, worsening in symptoms were observed in all patients over time. Our results corroborate the findings by Libik [31], who did not observe effects of PBM compared to the control group on dry mouth symptoms.

Due to worsening xerostomia and hyposalivation, patients have difficulties in swallowing, chewing and speaking [7]. In addition, all patients in this study developed radiation-induced mucositis at some point. These effects culminate in a worse quality of life [62]. Some studies have shown an improvement in quality of life of radiotherapy patients after PBM [63,64]. Despite this, our study found no effect of PBM on the quality of life of these patients. Part of this can be explained precisely by the fact that PBM did not modify xerostomia and hyposalivation, which are the main considerations that affect patients undergoing head and neck radiotherapy [7].

Limitations of the present study include the low sampling obtained due to the difficulty for patients to meet the inclusion and exclusion criteria. Even though this sample showed an increase in unstimulated salivary pH, a trend in increasing the pH of the stimulated saliva was visualized in LG at t3, but not significant. Perhaps in larger samples, the PBM may demonstrate a better effect on the stimulated salivary pH regulation. In addition, radiotherapy treatment is performed according to individual planning. Therefore, this study was unable to control the dose ($>$ 50Gy) and the distribution (uni or bilateral) of radiation in the major salivary glands, due to high heterogeneity found in patients. Moreover, the effects of PBM on salivary pH were observed especially during the laser application period. As it assessed short-term effects, this study did not demonstrate how long the PBM effect would last over the long term. Finally, there is a wide range of PBM protocols yielding different results. Our laser therapy protocol was developed by combining red and infrared wavelengths, as previously described in the literature [32,35,65]. The dosimetry of each wave was calculated by the average of total energy distributed according to previous studies [25,28,29,31–34]. Although it, our protocol showed no effect on salivary flow.

In conclusion, our PBM protocol was shown to increase the pH of unstimulated saliva at the end of radiotherapy of head and neck cancer patients. However, no improvement in quality of life, xerostomia, salivary flow and salivary composition could be observed in these patients. Due to limitations found in this study, future randomized

controlled clinical trials with larger samples and extended follow-up period are required to test new parameters of laser therapy in radiotherapy patients.

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Declaration of Competing Interest

The authors declare that they do not have a conflict of interest regarding the present study.

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